PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

13.11.05
13.12.05 per FD

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/001593	14.02.2005	13.02.2004

(22-Monats Frist)
im FB bez.

International Patent Classification (IPC) or both national classification and IPC
C12P19/26, C07K14/535, A61K38/17, G01N33/68

Applicant
GLYCOTOPE GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☐ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/001593

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: WO 03/016329 A (DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG DES OEFFENTLICHEN RECHTS; PA) 27 February 2003 (2003-02-27)

D2: VISWANATHAN KARTHIK ET AL: "Engineering sialic acid synthetic ability into insect cells: identifying metabolic bottlenecks and devising strategies to overcome them." BIOCHEMISTRY. 30 DEC 2003, vol. 42, no. 51, 30 December 2003 (2003-12-30), pages 15215-15225, XP002334628 ISSN: 0006-2960

D3: JACOBS C L ET AL: "Substrate specificity of the sialic acid biosynthetic pathway." BIOCHEMISTRY. 30 OCT 2001, vol. 40, no. 43, 30 October 2001 (2001-10-30), pages 12864-12874, XP002334629 ISSN: 0006-2960

D4: WO 00/52135 A (HUMAN GENOME SCIENCES, INC; JOHNS HOPKINS UNIVERSITY; UNIVERSITY OF WY) 8 September 2000 (2000-09-08)

D5: FUKUDA M ET AL: "Structures of novel sialylated O-linked oligosaccharides isolated from human erythrocyte glycophorins." THE JOURNAL OF BIOLOGICAL CHEMISTRY. 5 SEP 1987, vol. 262, no. 25, 5 September 1987 (1987-09-05), pages 11952-11957, XP002334630 ISSN: 0021-9258

The present application relates to a method of producing sialylated recombinant glycoproteins (e.g recombinant Granulocyte Macrophage Colony-Stimulating Factor) , using a host cell , that is deficient in UDP-GlcNac 2 epimerase, and that is supplemented with sialic acid analogues .

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty(Article 33.2 PCT)

D1 discloses the provision of glycoconjugates containing a sialic acid derivative used for immunosuppression, cell protection, stimulation of haematopoiesis, regulation of hormone secretion and hormonal activation. It discloses the BJA-B K20 and HL60-I host cells that are hyposialylated due to a UDP-GlcNAc 2-epimerase deficiency, a key enzyme of sialic acid biosynthesis. The fact that the hyposialylated cells have a defect in sialic acid biosynthesis makes them an ideal tool for the incorporation of modified sialic acid

precursors, as analogues do not need to compete with endogenously synthesized sialic acids. It was found that medium supplementation with NeuAc complemented for endogenous hyposialylation in BJA-B K20 and HL60-I cells. NeuAc was rapidly taken up, metabolized, incorporated into cellular glycoconjugates, and exposed at the cell surface . The glycoconjugates are obtained by conjugating a sialic acid derivate to a mono-, di- or oligosaccharide with up to 40 glycosidically linked, optionally branched sugar residues representing furanose and/or pyranose rings, which are linked N- or O-glycosidically to a polypeptide . (see the abstract , page 11 first paragraph- page 15 4th paragraph, claims 1-7 ,figs 1-6)

D2 discloses the engineering sialic acid synthetic ability into insect cells and related strategies to overcome them. It discloses the addition of the tetra-O-acetylated ManNAc which was easily taken up by the cells.(see the abstract)

D3 discloses the substrate specificity of the sialic acid biosynthetic pathway and the sialylation of glycoproteins . It discloses unnatural analogues of sialic acid can be delivered to mammalian cell surfaces through the metabolic transformation of unnatural N-acetylmannosamine (ManNAc) derivatives. The UDP-GlcNac 2 epimerase/ManNac-6 kinase is over expressed . The sialylated glycoprotein is secreted or delivered to the plasma membrane by the secretory machine . (see the abstract , page 12868 left column second paragraph , figs.1-9)

D4 discloses the recombinant production of sialylated glycoproteins using cells in which the expression of enzymes, e.g. sialic acid synthetase, involved in the sialylation reaction has been altered . It discloses a method for manipulating glycoprotein production in an insect cell, comprising enhancing expression of at least 1 enzyme selected from: GlcNAc-2 epimerase ,an enzyme catalyzing conversion of UDP-GlcNAc to ManNAc . E examples of proteins that benefit from the heterologous expression of the invention include, but are not limited to, transferrin, plasminogen, Na+, K+-ATPase, thyrotropin, tissue plasminogen activator, erythropoietin, interleukins, and interferons . (see the abstract , claims 26-45, and figs. 1-5,37)

D5 discloses the structures of novel sialylated O-linked oligosaccharides isolated from human erythrocyte glycophorins. In addition to the previously

described disialylated tetrasaccharide, NeuNAc alpha 2-3Gal beta 1-3 (Neu-NAc alpha 2-6)GalNAcOH and monosialylated trisaccharide, NeuNAc alpha 2-3Gal beta 1-3GalNAcOH, novel trisialylated oligosaccharides were isolated. (see the abstract and table II)

Claims 1-8 are defined as a product by process , without defining the exact technical features necessary to achieve the desired effect , and without defining the exact technical features necessary to discriminate unambiguously the claimed subject-matter from the prior art . Due to this , and view of D1-D5 the subject-matter of claims 1-23 is not new in the sense of art.33(2) PCT .

Re Item VIII

Certain observations on the international application

While claims 1-8 are defined as a product by process , without defining the exact technical features necessary to achieve the desired effect , and without defining the exact technical features necessary to discriminate unambiguously the claimed subject-matter from the prior art , its subject-matter is neither clear , nor deos it comprise all essential technical elements .

The terms 'the expression cell line NM-F9 or NM-D4' used in claim 6 are vague and ambiguous and leave the reader in doubt as to their exact technical meaning . The subject-matter of claim 6 lacks hence clarity .